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Chronic Toxicity and Bioaccumulation of Mercuric Chloride in the Fathead Minnow ('Pimephales promelas')

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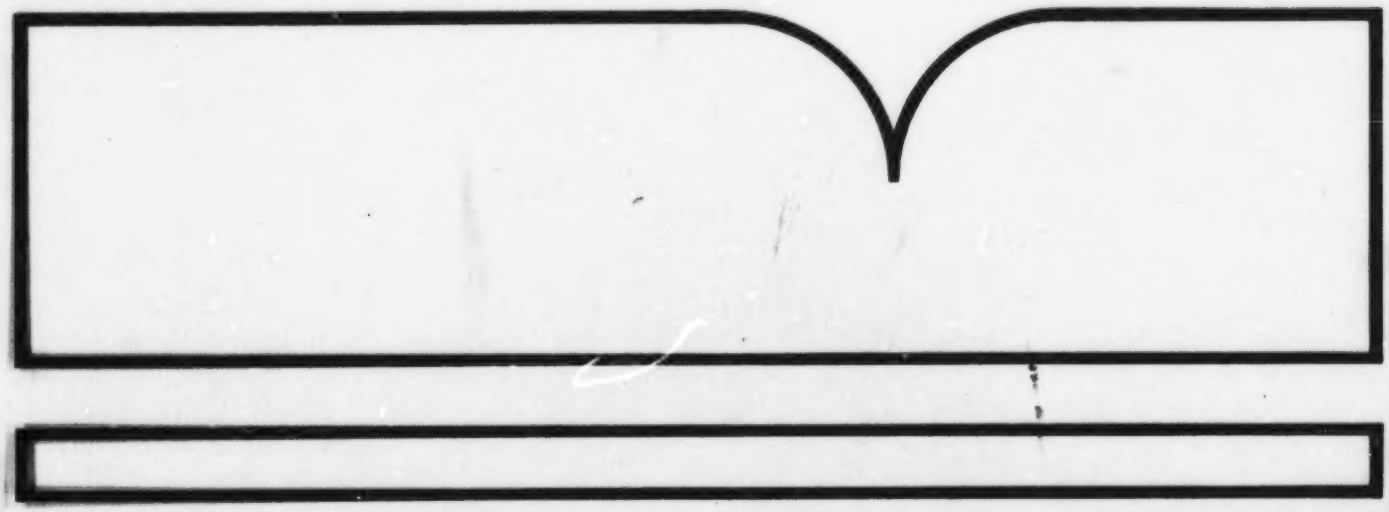

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16. ABSTRACT

Fathead minnows (Pimephales promelas) were exposed to various concentrations of mercuric chloride in water to determine its acute and chronic (including reproduction) toxicity and to measure bioaccumulation. Mean LC50 values of $HgCl_2$ for juvenile fatheads were 168, 112, 84, and 74 µg Hg/l at 4, 5, 6, and 7 days, respectively. Following 41 wk of exposure to 0.26 to 3.69 µg/l, females were significantly smaller than controls at all concentrations except 0.50 µg/l and males showed reduced growth at 3.69 µg/l. Over half of the fish at 2.01 and 3.69 µg/l were severely stunted and scoliosis was prevalent among these stunted fish. No spawning occurred at and above 1.02 µg/l and the number of spawning pairs was reduced at 0.26 and 0.50 µg/l resulting in total egg production of 46 and 54%, respectively, of control. Thirty-day growth of second-generation larvae (from transferred control embryos at and above 1.02 µg/l) was significantly reduced at all mercury concentrations tested. No effects on hatchability or larval survival was detected. Chronically-exposed fish accumulated mean whole body total mercury residues of 1.36 to 18.80 µg/g of tissue (wet weight) from 0.26 to 3.69 µg/l, respectively. Uptake appeared proportional to water concentration; bioconcentration factors varied between 4,380 and 5,680.

The influence of diet on mercury toxicity and bioaccumulation was studied during two 60-day larval fathead exposures. Overall reduced growth and possibly enhanced toxic effects occurred in larvae on the dry trout starter diet compared to larvae fed on Artemia. Mercury uptake appeared influenced by the severity of toxic effects.

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CHRONIC TOXICITY AND BIOACCUMULATION OF MERCURIC CHLORIDE IN THE FATHEAD MINNOW (*PIMEPHALES PROMELAS*)

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Fathead minnows (*Pimephales promelas*) were exposed to various concentrations of mercuric chloride in water to determine its acute and chronic (including reproduction) toxicity and to measure bioaccumulation. Mean LC_{50} values of $HgCl_2$ for juvenile fatheads were 168, 112, 84, and 74 μg Hg/l at 4, 5, 6, and 7 days, respectively. Following 41 wk of exposure to 0.26 to 3.69 μg/l, females were significantly smaller than controls at all concentrations except 0.50 μg/l and males showed reduced growth at 3.69 μg/l. Over half of the fish at 2.01 and 3.69 μg/l were severely stunted and scoliosis was prevalent among these stunted fish. No spawning occurred at and above 1.02 μg/l and the number of spawning pairs was reduced at 0.26 and 0.50 μg/l resulting in total egg production of 46 and 54%, respectively, of control. Thirty-day growth of second-generation larvae (from transferred control embryos at and above 1.02 μg/l) was significantly reduced at all mercury concentrations tested. No effects on hatchability or larval survival were detected. Chronically-exposed fish accumulated mean whole body total mercury residues of 1.36 to 18.80 μg/g of tissue (wet weight) from 0.26 to 3.69 μg/l, respectively. Uptake appeared proportional to water concentration; bioconcentration factors varied between 4380 and 5680.

The influence of diet on mercury toxicity and bioaccumulation was studied during two 60-day larval fathead exposures. Overall reduced growth and possibly enhanced toxic effects occurred in larvae on the dry trout starter diet compared to larvae fed on *Artemia*. Mercury uptake appeared influenced by the severity of toxic effects.

Key words: fathead minnows; mercuric chloride; acute and chronic toxicity; bioaccumulation; diet

INTRODUCTION

Mercury contamination of aquatic ecosystems is a serious and ecologically complex environmental problem. Mercury occurs in water in several organic and inorganic forms, depending on environmental variables. From a toxicological standpoint, methylmercury is considered the most important; but from a mass balance point of view, the inorganic forms, largely sediment bound and less available to the biota, predominate (Hartung and Dinman, 1972; Jernelöv et al., 1975). Changes in chemical form can occur through biological interconversions between organic and inorganic compounds (Jernelöv et al., 1975; Furutani et al.,

1980; Rudd and Furutani, 1980) and through changes in physicochemical environmental variables (Beijer and Jernelöv, 1979). The lowering of pH, for example (as when a river of neutral or slightly alkaline pH enters an acidified lake) can cause a release of free ion from complexes and particulate matter. Under such circumstances, mercuric ion would then be more biologically available and could become more toxicologically important.

Past studies, mostly involving short-term exposures, have shown that inorganic mercury bioaccumulates rapidly and is toxic to fish (MacLeod and Pessah, 1973; Kramer and Neidhart, 1975; Wobeser, 1975; Lorz, 1978). Matida (1971) studied mercury accumulation and toxicity in rainbow trout exposed to mercuric chloride for 14 wk; however, no studies involving continuous exposure of fish through all life stages have been published.

The present study was conducted to determine the acute and chronic (life cycle) toxicity of inorganic mercury (as mercuric chloride) to the fathead minnow (*Pimephales promelas* Rafinesque) and to measure the mercury residues accumulated in the whole body from the aqueous exposures. As part of this investigation, the influence of diet on mercury toxicity and residue accumulation also was studied.

MATERIALS AND METHODS

Exposure system

The flow-through exposure system consisted of a proportional diluter (Mount and Brungs, 1967) that delivered five mercury concentrations and a lake water control to duplicate 90 cm × 30.5 cm × 30.5 cm glass tanks, each receiving 500 ml/5 min cycle. During the spawning period, two 30.5 cm × 15.2 cm × 30.5 cm glass chambers with stainless steel mesh ends were placed in the rear third of each tank for larval exposure studies. Concurrently, the water supply to each tank was divided between these chambers and then flowed through the screen ends into the main tank. The 90% volume replacement times for the main tank and the larval chamber were approximately 14 h and 5.5 h, respectively (Sprague, 1969).

Unfiltered Lake Superior water was heated in a stainless steel headbox to 25± 2 °C before entering the diluter. Water temperatures were checked daily in the test tanks and ranged from 23–24 °C during the acute toxicity test and from 23–26 °C during the life-cycle tests. Mean (± SD) chemical characteristics of the test water, determined weekly according to standard methods (American Public Health Association et al., 1971), were total hardness, alkalinity, and acidity of 45.7 ± 1.0, 42.8 ± 2.1, and 3.1 ± 0.4 mg/l as $CaCO_3$, respectively; and pH of 7.2–7.5. Mean dissolved oxygen levels were 86 ± 5% saturation with a range of 62–98% over the entire study period.

Fluorescent lamps provided a light intensity of 30–50 ft-c at the water surface. A constant 16 h L:8 h D regime was used during the acute toxicity test while a simulated natural photoperiod was used during the longer experiments as recommended by U.S. EPA (1971).

Analytical procedures

A stock solution of mercuric chloride (Fisher Certified ACS) in de-ionized water was introduced into the diluter from a glass Mariotte bottle. Total mercury concentrations in water were analyzed by flameless atomic absorption according to the methods of Olson et al. (1975). All water concentrations are measured values reported as micrograms of mercury per liter (μg/l) and are given as mean ± SE in Tables I and II. During the acute exposure, water samples from all tanks were analyzed daily for total mercury. During the longer exposures, samples were analyzed for total mercury at least weekly from every tank and from in-flow delivery tubes of the 0.50 and 1.02 μg/l concentrations. Additional weekly samples from one duplicate tank at 0.50 and 1.02 μg/l were analyzed for total mercury and for inorganic mercury as described by Olson et al. (1975) to detect possible in-tank methylation. No statistical difference between total and inorganic mercury was detected in tank samples ($P = 0.05$), therefore we assumed very little methylation occurred in exposure tanks. Comparison of total mercury concentrations between the inflow and in-tank samples showed a mean loss of 23% mercury from both tanks over the exposure period. This mercury loss was probably due to formation of volatile metallic mercury (Hg^0). To maintain the desired in-tank mercury concentrations, the stock concentration was increased twice throughout the exposure period. Mean recovery of mercuric chloride added to control water at 0.50 or 1.00 μg Hg/l with each sample set was 99.9 ± 1.0% ($n = 60$).

Total mercury residues in the whole body samples were analyzed by the methods of Olson et al. (1975). Samples consisted of individual fish or composites of up to ten fish, depending on fish size and exposure concentration. Residue concentrations are expressed as μg Hg/g of tissue based on wet weight.

Biological exposures

Fathead minnows from our laboratory culture unit were used for these studies.

For the acute toxicity test, 17 fathead minnows, approximately 3 mth of age, were randomly distributed to each exposure tank and exposed to duplicate mean measured mercury concentrations of 0, 23, 48, 103, and 214 μg/l. Mortalities were recorded at regular intervals through 7 days.

Separate 60-day inorganic mercury exposures were run to study mercury toxicity and accumulation in fish maintained on different diets and to compare the relative suitability of a commercial dry food with a live food for testing larval fathead

minnows. At the start of each exposure, fifty 4- to 6-day-old fathead larvae were randomly distributed to each experimental tank. During the first experiment, the larvae were fed to satiation Glenco Mills trout chow starter 2 or 3 times daily. After 30 days of exposure, survival and growth (total length) were determined by the photographic method used by McKim and Benoit (1971). McKone et al. (1971) observed that a large portion of the mercury accumulated by goldfish from water was concentrated in the external mucus. Therefore, after 60 days, mercury-exposed fish were put into clean Lake Superior water for 1 h to remove surface mercury. All fish were killed, their weights and total lengths measured, and the number of deformed fish determined. Fish were homogenized and frozen for later residue analysis.

Two weeks later, a second experiment was begun in the same exposure system. These fish were fed live newly-hatched *Artemia* nauplii 4 times daily for 30 days then live nauplii and frozen adult *Artemia* 4 times daily for the next 30 days. Survival and growth data were collected as in the first experiment except at 60 days 15 fish were randomly selected from each tank to continue through a reproductive cycle. The remaining fish were killed, measured, and frozen for residue analyses as previously described.

Throughout the remainder of the chronic exposure, fish were fed frozen *Artemia* 3 times daily supplemented with live laboratory-cultured mixed zooplankton (mostly cladocerans and copepods) 4 times weekly.

On day 143 of exposure the male and female fish in the control and 3 lowest mercury concentrations were paired to obtain information on individual reproductive performance. With the larval chambers in the rear third of each tank, stainless steel mesh was used to divide the remaining area into 4 compartments and one pair of minnows was placed into each with one spawning tile. The fish in the two highest mercury concentrations were not thinned to 8 fish/tank because they were considerably smaller, extremely variable in size, and showed no visible sexual dimorphism.

Test procedures during the spawning period were those recommended by U.S. EPA (1971) with spawning activity recorded daily and hatchability determined by incubating 50 embryos per spawn at the concentration in which they were spawned. Upon hatching, 20 larvae from selected incubations were transferred to larval chambers for early life-stage studies. Larvae were fed live *Artemia* nauplii as previously described. Survival, total lengths, and weights were determined after 30 days of exposure. In tanks where no spawning occurred, control embryos were transferred when < 24 h old to obtain early life-stage information at these concentrations.

Following a 4-mth spawning period, spawning was terminated by gradually decreasing photoperiod. The fish were killed when 1 wk had elapsed since the last spawning as recommended by U.S. EPA (1971). Total length and weight of each fish were measured and individual homogenates prepared for residue analyses.

Statistical analyses

All data were analyzed by Student's *t*-test for differences between replicates. No significant differences were detected ($P = 0.05$); therefore, replicate data were combined. LC_{50} values were computed from the acute toxicity data by the trimmed Spearman–Karber method (Hamilton et al., 1977). Survival, growth, deformity, spawning, and hatchability data were analyzed by one-way analysis of variance (with arcsin transformation of proportion data) and Dunnett's procedure (Steel and Torrie, 1960).

RESULTS

Acute and chronic toxicity

Mean LC_{50} values (95% CI) of $HgCl_2$ for juvenile fathead minnows were 168 (100–282), 112 (76–164), 84 (68–104), and 74 (62–90) μg/l after 4, 5, 6, and 7 days, respectively.

Results of the 60-day mercuric chloride exposure of fatheads fed dry trout starter are presented in Table I. Survival was significantly reduced compared to the control only at the highest concentration, 4.51 μg/l ($P < 0.01$). Most of the mortality occurred within the first 30 days of exposure. After 30 days of exposure significant growth retardation was seen at and above 1.27 μg/l. After 60 days fish at 0.58 μg/l were also significantly smaller (less in total length and weight) than control fish ($P < 0.05$). An increasing incidence of spinal curvature was seen with increasing mercury concentration among survivors at 60 days; however, this teratogenic effect was statistically different from controls only at the highest exposure level, 4.51 μg/l.

Results of the 60-day exposure of *Artemia*-fed fish are presented in Table II. Mean mercury concentrations during this exposure were 15–20% lower than during the dry trout food experiment. No significant difference in survival was seen at any mercury concentration ($P > 0.05$). After 30 days exposure, fish at and above 0.50 μg/l were significantly shorter than the controls ($P < 0.05$). However, after 60 days, only those at 2.01 and 3.69 μg/l were significantly shorter and lighter in weight than controls ($P < 0.05$). No deformed fish were observed at 60 days.

Comparison of the two 60-day exposures (Tables I and II) reveals little difference in survival not attributable to mercury exposure. Control survival at 60 days was 94% and 96% for dry trout starter- and *Artemia*-fed fish, respectively. However, growth was noticeably improved on the *Artemia* diet among all experimental groups. *Artemia*-fed fish were also more uniform in size than those on the trout starter, particularly in control and the lower mercury concentrations. For example, the relative standard deviations (RSD = standard deviation expressed as a percent of the mean) for 60-day lengths and weights were 12.8 and 41.6%, respectively, for

TABLE I

Thirty- and 60-day survival and growth of dry trout starter-fed fathead minnows exposed to various water concentrations of $HgCl_2$. Replicates combined.

	$HgCl_2$ concentration ($\bar{X}$ ± SE as μg Hg/l)					
	Control (< 0.01)	0.31 ± 0.05	0.58 ± 0.09	1.27 ± 0.16	2.43 ± 0.34	4.51 ± 0.62
30 Days						
% survival	94	89	86	76	73	52[a]
Total length (mm)	16.5 ± 3.5	17.5 ± 2.5	16.0 ± 3.0	15.0[b] ± 3.5	14.5[b] ± 2.5	12.0[b] ± 2.5
± SD (*N*)	(91)	(89)	(86)	(73)	(73)	(52)
60 Days						
% survival	94	88	83	73	72	50[a]
Total length (mm)	28.5 ± 5.0	27.5 ± 4.0	25.0[b] ± 3.5	24.0[b] ± 4.5	22.0[b] ± 4.0	23.0[b] ± 5.0
± SD (*N*)	(94)	(88)	(83)	(73)	(72)	(50)
Weight (g)	0.224 ± 0.147	0.202 ± 0.103	0.134[b] ± 0.062	0.143[b] ± 0.082	0.131[b] ± 0.072	0.131[b] ± 0.090
± SD (*N*)	(94)	(76)	(82)	(70)	(71)	(47)
Incidence of spinal curvature	2/94	3/88	7/83	7/73	10/72	13/50[c]
% deformed	2.1	3.4	8.4	9.6	13.9	26.0

[a]Significantly less than control, $P < 0.01$.
[b]Significantly less than control, $P < 0.05$.
[c]Significantly greater than control, $P < 0.05$.

TABLE II

Thirty- and 60-day survival and growth of *Artemia*-fed fathead minnows exposed to various water concentrations of $HgCl_2$. Replicates combined.

	$HgCl_2$ concentration ($\bar{X}$ ± SE as μg Hg/l)					
	Control (< 0.01)	0.26 ± 0.05	0.50 ± 0.08	1.02 ± 0.14	2.01 ± 0.28	3.69 ± 0.57
30 Days						
% survival	96	92	93	78	94	83
Total length (mm)	31.0 ± 3.5	31.0 ± 2.5	30.0[a] ± 2.5	30.0[a] ± 2.5	26.0[a] ± 3.0	18.5[a] ± 3.0
± SD (*N*)	(90)	(88)	(92)	(77)	(89)	(81)
60 Days						
% survival	96	92	93	78	94	74
Total length (mm)	44.5 ± 5.5	45.5 ± 5.5	43.5 ± 5.0	44.5 ± 6.0	38.0[a] ± 5.5	27.5[a] ± 5.5
± SD (*N*)	(64)	(62)	(63)	(48)	(64)	(44)
Weight (g)	1.011 ± 0.421	1.040 ± 0.412	0.927 ± 0.362	0.926 ± 0.411	0.541[a] ± 0.232	0.210[a] ± 0.156
± SD (*N*)	(64)	(62)	(63)	(48)	(64)	(44)

[a]Significantly less than control, $P < 0.05$.

Artemia-fed controls and 18.0 and 65.6%, respectively, for starter-fed control fish.

Growth (weight only) and reproduction data from the chronically-exposed fish and survival and growth data for the second generation fish are presented in Table III. No statistical differences in survival were detected at any mercury concentration during the 41-wk chronic exposure. Significant growth retardation was detected at 3.69 μg/l among males and at all mercury concentrations except 0.50 μg/l among females after 41 wk of continuous exposure ($P < 0.05$). At 2.01 and 3.69 μg/l gonads of many of the smallest fish were undeveloped and sexes could not be accurately distinguished by gross autopsy. These fish have been designated as 'undeveloped' in Table III. Among these 'undeveloped' fish, scoliosis (lateral spinal curvature) occurred in 2 of 11 (18%) and 5 of 13 (38%) at 2.01 and 3.69 μg/l, respectively.

Spawning data show significant reproductive impairment of chronically-exposed fathead minnows. No spawning occurred in the 3 highest mercury concentrations; in fact, no females were mature in either 1.02 or 3.69 μg/l at termination. However, 3 of 5 females at 2.01 μg/l had at least partially developed ovaries. During the 4-mth spawning period, all 8 control females spawned but only 3 at 0.26 μg/l and 6 at 0.50 μg/l spawned. Total egg production at 0.26 and 0.50 μg/l was 46 and 54%, respectively, of control. The number of eggs spawned per female varied considerably at each concentration (ranges given) and no significant differences were seen in either mean eggs per spawning female or mean eggs per spawn ($P > 0.05$).

Hatchability and larval survival at 30 days post-hatch was not significantly affected at any mercury concentration either from embryos incubated at parental exposure concentrations or from embryos transferred from control and incubated at concentrations where no spawning occurred. However, growth of larval fish was reduced. After 30 days of exposure, second generation larvae at all mercury concentrations were significantly lighter and shorter than controls ($P < 0.01$).

Residue accumulation

Results of the residue analyses are summarized in Table IV. After 60 days of exposure fathead minnows fed dry trout starter accumulated mean whole body mercury concentrations ranging from 0.80 μg/g at the lowest water concentration (0.31 μg/l) to 4.18 μg/g at the highest concentration (4.51 μg/l). Although the residues increased with increasing exposure concentration, the relationship was nonlinear: bioconcentration factors (BCFs) decreased with increasing water concentrations from 2580 to 930 for the lowest to the highest water concentrations, respectively. Exposed fish maintained on the *Artemia* diet accumulated mean whole body residues after 60 days ranging from 0.62 μg/g at the lowest concentration (0.26 μg/l) to 7.60 μg/g at 3.69 μg/l. In contrast with the dry-food experiment, uptake appeared linear over the exposure range; BCFs varied between 2060 (at 3.69 μg/l)

TABLE III

Growth and reproduction of fathead minnows after 41 wk of exposure to mercuric chloride and survival and growth of their progeny after 30 days of exposure. Replicates combined.

	$HgCl_2$ concentration (as μg Hg/l)					
	Control (< 0.01)	0.26	0.50	1.02	2.01	3.69
Chronically exposed fish						
Weight (g) $\bar{X}$ ± SD (*N*)						
Males	5.38 ± 0.97 (8)	5.75 ± 1.18 (8)	5.52 ± 1.00 (7)	6.23 ± 0.71 (7)	4.41 ± 2.29 (8)	3.01[a] ± 0.66 (4)
Females	2.32 ± 0.26 (8)	1.90[a] ± 0.36 (7)	2.08 ± 0.20 (8)	1.70[b] ± 0.37 (8)	1.56[a] ± 0.40 (5)	–
Undeveloped[c]					0.84[d] ± 0.29 (11)	0.96[e] ± 0.42 (13)
Proportion of females that spawned	8/8	3/8	6/8	0/8	0/5	–
Mean eggs per spawning female (range)	1204 (90–2204)	1485 (365–2590)	861 (180–1483)	–	–	–
Mean eggs per spawn	214	223	198	–	–	–
Total eggs spawned	9635	4455	5165	0	0	0
Mean % hatch	83	60	78	91[g]	88[g]	85[g]
(*N* of replicates)[f]	(32)	(18)	(16)	(4)	(4)	(5)
Progeny after 30 days of exposure						
% survival	87	50	85	50[g]	85[g]	70[g]
Length (mm) $\bar{X}$ ± SD (*N*)	30.0 ± 2.5 (87)	28.5[b] ± 1.0 (30)	29.0[b] ± 2.0 (51)	27.5[b] ± 2.0 (20)	25.0[b] ± 2.5 (51)	21.5[b] ± 2.5 (28)
Weight (g) $\bar{X}$ ± SD (*N*)	0.261 ± 0.058 (87)	0.192[b] ± 0.026 (30)	0.233[b] ± 0.055 (51)	0.192[b] ± 0.041 (20)	0.153[b] ± 0.045 (51)	0.090[b] ± 0.024 (28)

[a] Significantly less than control, $P < 0.05$.
[b] Significantly less than control, $P < 0.01$.
[c] Sex could not be distinguished by gross autopsy.
[d] Two of 11 exhibited scoliosis.
[e] Five of 13 exhibited scoliosis.
[f] Replicate = group of 50 embryos incubated from one spawn.
[g] Transferred from control as embryos < 24 h after spawning.

and 2590 (at 1.02 μg/l). After 41 wk of mercuric chloride exposure, mean whole body residues were approximately double those at 60 days, ranging from 1.36 μg/g to 18.80 μg/g from the lowest to the highest exposure levels. BCFs suggest that uptake was directly proportional to water concentration.

DISCUSSION

Inorganic mercury, added as $HgCl_2$, is highly toxic to the fathead minnow with low μg/l water concentrations resulting in decreased survival, spinal deformities, reduced growth and impaired reproduction.

The 4-day (96-h) LC_{50} value for juvenile fathead minnows of 168 μg/l in this study is similar to acute toxicity values of mercuric chloride reported for other freshwater fish. MacLeod and Pessah (1973) reported 96-h LC_{50} values for juvenile rainbow trout (*Salmo gairdneri*) of 400, 280, and 220 μg/l at 5, 10, and 20 °C. Also with $HgCl_2$ and rainbow trout, Matida et al. (1971) obtained a 96-h LC_{50} of 210 μg/l at 16.7 °C and Wobeser (1975) reported a 24-h LC_{50} of 903 μg/l at 10 °C. Lorz (1978) reported a 96-h LC_{50} of 240 μg/l for juvenile coho salmon (*Oncorhynchus kisutch*). Hanumante and Kulkarni (1979) reported a 96-h LC_{50} of 1400 μg/l for the Indian food fish, *Channa gachua*. The present study showed that chemical exposure to mercuric chloride reduces survival well below these acute values with only 52% of larval fatheads surviving 30 days of exposure to 4.51 μg/l.

In this study, a significantly higher incidence of spinal curvatures occurred among the dry trout starter-fed fathead minnows at 4.51 μg/l. This deformity also was noted among the severely stunted fish chronically exposed to 2.01 and 3.69 μg/l. Teratogenic and embryotoxic effects of $HgCl_2$ to teleosts have been previously reported. Heisinger and Green (1975) reported reduced hatch, inability to uncurl, and other abnormalities in Japanese medaka, *Oryzias latipes*, following embryonic exposure to 15 μg Hg/l. Weis and Weis (1977) observed developmental anomalies, including reduced axis formations and forebrain defects, among *Fundulus heteroclitus* embryos exposed to 10 to 1000 μg Hg/l as $HgCl_2$ and reported retarded growth among embryos that did not exhibit malformations. Recently, studying this same marine killifish, Sharp and Neff (1980) found reduced hatching success at and above 20 μg Hg/l and increased incidence of lateral spinal curvature at and above 30 μg Hg/l among eleutheroembryos emerging following chronic embryonic exposure (32 days).

Growth and reproduction were even more sensitive indicators of the direct toxicity of inorganic mercury. Matida et al. (1971) observed retarded growth of juvenile rainbow trout exposed to $HgCl_2$ in water at 21 μg/l and reported a permissible concentration range, based on growth, between 2.1 and 21 μg/l. In the present study, mercury exposure at all concentrations tested, 0.26 to 3.69 μg/l, reduced growth of second generation larvae. Among the first generation fish that

TABLE IV

Whole body concentrations of total mercury in fathead minnows fed different diets during exposure to $HgCl_2$ in water.

Mean exposure concentration (μg Hg/l)	Diet	Mercury residues, μg/g of tissue (wet weight)					
		60-Day exposure			41-wk exposure		
		$\bar{X}$ ± SE	N samples (fish per sample)	BCF	$\bar{X}$ ± SE	N samples (fish per sample)	BCF
< 0.01 (control)	Dry trout starter	0.12 ± 0.01	6 (1–3)	–	– [a]	–	–
0.31	Dry trout starter	0.80 ± 0.04	6 (1–2)	2580	–	–	–
0.58	Dry trout starter	1.31 ± 0.04	6 (1–2)	2260	–	–	–
1.27	Dry trout starter	1.98 ± 0.10	6 (1)	1560	–	–	–
2.43	Dry trout starter	2.75 ± 0.20	6 (1)	1130	–	–	–
4.51	Dry trout starter	4.18 ± 0.15	6 (1)	930	–	–	–
< 0.01 (control)	*Artemia*	0.22 ± 0.01	6 (5)	–	0.32 ± 0.01	5 (1)	–
0.26	*Artemia*	0.62 ± 0.03	6 (5)	2390	1.36 ± 0.04	8 (1)	5230
0.50	*Artemia*	1.24 ± 0.06	6 (5–6)	2480	2.84 ± 0.13	6 (1)	5680
1.02	*Artemia*	2.64 ± 0.08	6 (5)	2590	4.47 ± 0.19	8 (1)	4380
2.01	*Artemia*	4.76 ± 0.17	6 (6–10)	2370	9.41 ± 0.67	6 (1)	4680
3.69	*Artemia*	7.60 ± 0.36	5 (5–9)	2060	18.80 ± 2.34	4 (1)	5100

[a] Test terminated after 60 days.

were 4–6 days old at the initiation of exposure, growth retardation was demonstrated as low as 0.50 μg/l during the first 60 days of exposure. Following 41 wk of continuous exposure, females were significantly smaller at all concentrations except 0.50 μg/l (the next to lowest concentration) while males were significantly smaller only at 3.69 μg/l. Part of this apparent differential sensitivity to mercury by sex could reflect the nonrandom prespawning thinning when males showing obvious secondary sexual characteristics, probably the largest and fastest growing individuals, were selected.

Reproductive inhibition of chronically-exposed fish was observed at all mercury concentrations tested. This effect on reproduction resulted mainly from the failure of some mercury-exposed fish to mature and spawn as no differences were detected in either the size of the spawns or the mean eggs per female that spawned. Reproductive impairment was seen in a previous $HgCl_2$-fathead minnow chronic exposure (Snarski and Olson, unpubl. data) where spawning activity occurred sporadically only among the three lowest concentrations, ranging from 0.75 to 2.75 μg/l. At 0.75 μg/l the total eggs spawned was only 40% of the control; at 1.50 μg/l no spawning occurred; and at 2.75 μg/l spawning occurred in only one duplicate tank, total eggs spawned was only 5% of the control, and the mean spawn size was reduced to only 30% of the control (69 eggs vs. 209 eggs).

While inorganic mercury generally has been considered less toxic than methylmercury, the chronic toxicity of $HgCl_2$ to fathead minnows reported here compares to the chronic toxicity of methylmercuric chloride (MMC) to brook trout (*Salvelinus fontinalis*) reported by McKim et al. (1976). Their maximum acceptable toxicant concentration (MATC) of MMC for brook trout was between 0.29 and 0.93 μg/l. At a MMC concentration of 0.93 μg/l, second-generation trout developed deformities and all but one female died during spawning. Survival, growth, and reproduction were not affected at 0.29 μg/l over the 3-generation study. In our study, the MATC of $HgCl_2$ for fathead minnows is < 0.26 μg/l, our lowest mercury concentration.

Diet appears to indirectly influence residue levels by influencing mercury toxicity. Bioconcentration factors appeared inversely related to the severity of the toxic effects. Similar BCFs at the lowest concentrations in both 60-day experiments showed that uptake was proportional to water concentration even though *Artemia*-fed fish weighed 4 to 6 times more than trout starter-fed fish. In the dry trout starter experiment, BCFs decreased with increased water concentration and growth was progressively reduced beginning at 0.58 μg/l, the lowest observed effect concentration (LOEC), up to 4.51 μg/l, where both growth and survival were affected. In the *Artemia* experiment, toxic growth reduction at 60 days was first detected at 2.01 μg/l and BCFs were generally similar with only the BCF at the high concentration slightly lower (2060 vs. 2370–2590). Possibly the dry starter diet was nutritionally deficient and enhanced the mercury toxicity. Or, alternatively, the richer *Artemia* diet may have allowed the fish to compensate for the toxic effects as

the fish reached a less sensitive life stage while the smaller fish, on the more marginal trout starter diet, were unable to overcome the initial growth suppression. Limited data tend to support the second interpretation. An apparent drop in sensitivity of *Artemia*-fed fish occurred between 30 and 60 days with the LOEC changing from 0.50 μg/l to 2.01 μg/l. Wobeser (1975) previously reported an increase in relative resistance to mercury compounds with increasing age and size of rainbow trout. Nutritional inadequacy of the dry starter diet for larval fatheads may also have contributed to the development of terata at 60 days that were not observed at 60 days among *Artemia*-fed larvae exposed to mercury.

Mean whole body mercury residues after 41 wk of exposure of 1.36 to 8.80 μg/g were approximately double those after 60 days of exposure. BCFs from 4380 to 5230 indicated that residue levels were generally proportional to water concentration. Olson et al. (1975) reported whole body total mercury residues in fathead minnows of 1.47 to 10.90 μg/g following 48 wk of exposure to methylmercury water concentrations of 0.018 to 0.247 μg/l, respectively. BCFs decreased from 8.2×10^4 to 4.4×10^4 for the lowest to highest water concentration, respectively. These two studies showed the uptake of mercury to be from 8.5 to 16 times higher from methylmercury exposure than from Hg^{2+} exposure. Others have also reported slower and lesser overall uptake of mercury from inorganic exposure compared to methylmercury exposure (Matida et al., 1971; Kramer and Neidhart, 1975).

The present study shows mercuric chloride to be toxic and substantially bioaccumulated in fish exposed to water concentrations as low as 0.26 μg/l over an extended period. These findings suggest the potential danger to fish populations if ionic mercury is released from contaminated sediments by changing environmental variables such as lowered pH associated with acid precipitation.

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REFERENCES

American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971. Standard methods for the examination of water and wastewater. 13th ed., New York, NY, 874 pp.

Beijer, K. and A. Jernelöv, 1979. Sources, transport and transformation of metals in the environment. In: Handbook on the toxicology of metals, edited by L. Friberg, G.F. Nordberg and V.B. Vouk, Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 47–63.

Furutani, A. and J.W.M. Rudd, 1980. Measurement of mercury methylation in lake water and sediment samples. Appl. Environ. Microbiol. 40, 770–776.

Hamilton, M.A., R.C. Russo and R.V. Thurston, 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11, 714–718.

Hanumante, M.M. and S.S. Kulkarni, 1979. Acute toxicity of two molluscicides, mercuric chloride and pentachlorophenol, to a freshwater fish (*Channa gachua*). Bull. Environ. Contam. Toxicol. 23, 725–727.

Hartung, R. and D.B. Dinman, eds., 1972. Environmental mercury contamination. Ann Arbor Sci. Publ. Inc., Ann Arbor, MI, p. 198.

Heisinger, J.F. and W. Green, 1975. Mercuric chloride uptake by eggs of the ricefish and resulting teratogenic effects. Bull. Environ. Contam. Toxicol. 14, 665–673.

Jernelöv, A., L. Landner and T. Larsson, 1975. Swedish perspectives on mercury pollution. J. Water Pollut. Control Fed. 47, 810–822.

Kramer, H.J. and B. Neidhart, 1975. The behavior of mercury in the system water-fish. Bull. Environ. Contam. Toxicol. 14, 699–704.

Lorz. H.W., 1978. Effects of several metals on smolting of coho salmon. U.S. Environ. Prot. Agency, Corvallis, OR, EPA-600/3-78-090, 84 pp.

MacLeod, J.C. and E. Pessah, 1973. Temperature effects on mercury accumulation, toxicity, and metabolic rate in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30, 485–492.

Matida, Y., H. Kumada, S. Kimura, Y. Saiga, T. Nose, M. Yokote and H. Kawatsu, 1971. Toxicity of mercury compounds to aquatic organisms and accumulation of the compounds to aquatic organisms and accumulation of the compounds by the organisms. Bull. Freshwater Fish. Res. Lab. 21, 197–227.

McKim, J.M. and D.A. Benoit, 1971. Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 28, 655–662.

McKim, J.M., G.F. Olson, G.W. Holcombe and E.P. Hunt, 1976. Long-term effects of methylmercuric chloride on three generations of brook trout (*Salvelinus fontinalis*): Toxicity, accumulation, distribution, and elimination. J. Fish. Res. Board Can. 33, 2726–2739.

McKone, C.E., R.G. Young, C.A. Bache and D.J. Lisk, 1971. Rapid uptake of mercuric ion by goldfish. Environ. Sci. Tech. 5, 1138–1139.

Mount, D.I. and W.A. Brungs, 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1, 21–29.

Olson, G.F., D.I. Mount, V.M. Snarski and T.W. Thorslund, 1975. Mercury residues in fathead minnows, *Pimephales promelas* Rafinesque, chronically exposed to methylmercury in water. Bull. Environ. Contam. Toxicol. 14, 129–134.

Rudd, J.W. M., A. Furutani and M.A. Turner, 1980. Mercury methylation by fish intestinal contents. Appl. Environ. Microbiol. 40, 777–782.

Sharp, J.R. and J.M. Neff, 1980. Effects of the duration of exposure to mercuric chloride on the embryogenesis of the estuarine teleost, *Fundulus heteroclitus*. Mar. Environ. Res. 3, 195–213.

Sprague, J.B., 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3, 793–821.

Steel, R.G.D. and J.H. Torrie, 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York, NY, 481 pp.

U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, 1971. Recommended bioassay procedure for fathead minnow *Pimephales promelas* Rafinesque chronic tests. (Revised 1972), Duluth, MN, 13 pp.

Weis, J.S. and P. Weis, 1977. Effects of heavy metals on development of the killifish, *Fundulus heteroclitus*. J. Fish. Biol. 11, 49–54.

Wobeser, G., 1975. Acute toxicity of methyl mercury chloride and mercuric chloride for rainbow trout (*Salmo gairdneri*) fry and fingerlings. J. Fish. Res. Board Can. 32, 2005–2013.